RECEIVED
CENTRAL FAX CENTER
AUG 22 2007

VPI/02-128 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Cecilia C. Chang
Group	:	1625
Applicants	:	Paul S. Charifson et al.
Serial No.	:	10/767,638
Confirmation No.	:	5416
Filed	:	January 29, 2004
For	:	GYRASE INHIBITORS AND USES THEREOF

Cambridge, Massachusetts
August 22, 2007

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PAUL S. CHARIFSON UNDER 37 C.F.R. § 1.132

I, PAUL S. CHARIFSON, a citizen of the United States of America, residing at 7 Dartmouth Drive, Framingham, MA (US), hereby declare that:

1. I am one of the named inventors of the above-identified patent application.

2. I received a B.S. in Pharmacy from the University of Rhode Island, Kingston, RI in 1983. In 1988 I received a Ph.D. in Medicinal Chemistry from the University of North Carolina, Chapel Hill, NC. After receiving my Ph.D., I was a Post-Doctoral Research Associate at the University of North Carolina in Chapel Hill from 1988-1990. From 1990 to 1991, I was employed as a computational chemist at Cray Research, Inc. From 1991 to 1996, I was employed as a Senior Research Scientist at Glaxo Wellcome, Inc. In 1997, I joined

Vertex Pharmaceuticals, Inc. (hereinafter "Vertex"). I have published 37 scientific papers in peer-reviewed journals. A copy of my curriculum vitae is attached as Exhibit B.

3. From 1999 until 2005, I was the Project Leader for the Bacterial Topoisomerase drug discovery team at Vertex. My work during 1999 to 2005 was devoted to the design and evaluation of gyrase B/topoisomerase IV inhibitors as antibacterials to treat human bacterial diseases. I have co-authored four papers and have been a co-inventor on two issued U.S. patents and nine U.S. patent applications directed to the Gyrase B enzyme and Gyrase B inhibitors.

4. I am familiar with the February 22, 2007 Office Action (hereinafter "Office Action") in the above-identified application. I understand that, in the Examiner's view, claims 26-29 of the present invention are rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. Specifically, the Examiner contends that "there is limited disclosure of the instantly claimed compounds being tested positive in an *S. aureus* MIC assay." The Examiner further contends that "no enablement support for this one bacteria activity can be extrapolated to broad spectrum "antibacterial" activity." See page 3, ¶ 3 of the Office Action.

5. I make this declaration for the following purposes:

a) To describe how the MIC assay, used in Vertex's laboratories, is the most widely used and accepted *in vitro* indicator of a compound's ability to kill bacterial strains of interest. See ¶ 6.

b) To provide *in vitro* minimum inhibitory concentration (hereinafter "MIC") assay results for the bacterial organisms *S. aureus*, *S. pneumoniae*, and *E. faecalis* for assayed compounds of the present invention. The *S. aureus*, *S. pneumoniae*, and *E. faecalis* MIC data demonstrates that the compounds of the present invention have Gram-positive activity against these representative Gram-positive species and are effective antibacterial agents generally for Gram-positive bacteria. See ¶¶ 7 and 8.

c) To provide additional rationale to support applicants' claim that one skilled in the art would expect that the claimed gyrase inhibitors would be effective for treating Gram-positive bacterial infections, particularly *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Coag. Neg. Staph*, *Bacillus anthracis*, and *Staphylococcus epidermidis*. See ¶¶ 9 and 10.

6. The MIC assays were run according to the Clinical and Laboratory Standards Institute (hereinafter "NCCLS") standardized MIC assay procedure provided in the specification in Example 29 (see, paragraphs [00152] to [00153] at pages 84-85). The MIC assay measures the lowest concentration of an antimicrobial agent that prevents viable growth of a microorganism in an agar or broth dilution susceptibility test. The methodologies for determining the MIC of a particular compound for a particular bacterial species are standardized and recognized as a reliable indicator of how effectively a compound inhibits the growth of bacteria in culture. In other words, the MIC of a compound is a metric of its potency against a particular bacterial strain. The NCCLS (http://www.nccls.org/) is a widely recognized, global, nonprofit organization that develops, publishes and implements standards for susceptibility testing and has developed such standards for determining and reporting MIC data. These MIC standards and assay methods are used throughout the industry in the development of antibacterial compounds. Our laboratories use these NCCLS standardized MIC assay methods for determining the *in vitro* antimicrobial activity of our gyrase inhibitors.

7. I have attached hereto Exhibit A, Table 1A, providing MIC data against the bacterial organisms *S. aureus*, *S. pneumoniae*, and *E. faecalis* for the compound species that were tested in each of these MIC assays. For the *S. aureus* and *E. faecalis* MIC assay, those compounds include numbers I-1 to I-32, I-37 to I-41, I-45 to I-57, I-60 to I-65, I-68 to I-283, I-285 and I-288 to I-295 of the present invention (282 compounds total, see specification, Table 2 at pages 18-37). For the *S. pneumoniae* MIC assay, those compounds include all those listed above except for compounds I-8, I-9, I-12, and I-16.

8. The MIC antibacterial assays against the Gram-positive organisms *S. aureus*, *S. pneumoniae*, and *E. faecalis* for the Gyrase B inhibitors shown in Table 1A in Exhibit A, were run according to the methods described in the specification in Example 29 at pages 84-85, paragraphs [00152] to [00153]. The MIC data for these 282 representative compounds showed bactericidal activity against these three Gram-positive bacteria. For instance, 221 of 282 (78%) compounds exhibited MIC's against *S. aureus* of less than 0.50 ug/ml; 248 of 282 (88%) compounds exhibited MIC's against *E. faecalis* of less than 0.50 ug/ml; and 263 of 278 (95%) compounds exhibited MIC's against *S. pneumoniae* of less than 0.50 ug/ml.

9. I also provide the following scientific rationale to explain why one of skill in the antimicrobial arts would reasonably expect that a compound with demonstrated activity against three Gram-positive organisms (e.g., *S. aureus*, *S. pneumoniae*, and *E. faecalis*) would also be expected to be active against most other Gram-positive organisms. First, the gyrase and topoisomerase IV enzymes are similar amongst all the Gram-positive bacterial organisms. Given the high homology and conserved active sites for these enzymes, one would expect that a compound that bound tightly to a bacterial gyrase and Topoisomerase IV for one Gram-positive bacteria, would also bind in a similar manner to these two enzymes from other Gram-positive bacteria. Currently approved antibiotics that target gyrase and/or topoisomerase IV and other classes of antimicrobials that bind to other targets support this observation. For example, the FDA-approved gyrase inhibitor, Novobiocin, exhibits activity in most Gram-positive organisms. Despite Novobiocin's structural dissimilarity to the compounds of the present invention, both inhibitor classes bind in the same ATP binding site of Gyrase B. Hence one would expect, and indeed does observe, similar Gram-positive activity for both of these inhibitor classes. Similarly, the potent gyrase/topoisomerase IV fluoroquinolone class of inhibitors (that bind to the A subunit of Gyrase and to the C subunit of topoisomerase IV, e.g., Ciprofloxacin) inhibits a broad range of Gram-positive bacteria. Likewise, other antibiotic classes that inhibit different bacterial enzymes, such as penicillins, beta-lactams, cephalosporins, and macrolides generally have activity against a host of Gram-positive organisms. Second, most Gram-positive organisms have similar cell wall and membrane structures and therefore have similar permeability properties and susceptibility to antimicrobial agents. Thus, one skilled in the art would conclude that a Gram-positive inhibitor would have antibacterial activity against most, if not all, Gram-positive bacterial organisms. Finally, Gram-positive organisms possess no additional gyrase or topoisomerase enzymes. Thus, if either or both of these essential enzymes are inhibited, bacterial organisms will have no fall-back mechanism to replicate their DNA and subsequently their cell growth and division will be halted.

10. Therefore, for the reasons presented above in ¶¶ 5-9 and the MIC data presented herewith in Exhibit A, it is my opinion that a skilled artisan would reasonably believe that the claimed compounds of the present invention were enabled for antibacterial activity against Gram positive bacteria, particularly the nine species recited in the specification.

11. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Paul S. Charifson

Paul S. Charifson

Signed this 22nd day of August, 2007
At Cambridge, MA, USA.